

AD_____

Award Number: DAMD17-97-1-7162

TITLE: Induction of Apoptosis in Human Breast Cancer by
Adenoviral-Mediated Gene Transfer of the Transcription
Factor E2F-1

PRINCIPAL INVESTIGATOR: Kelly Hunt, M.D.

CONTRACTING ORGANIZATION: The University of Texas M.D.
Anderson Cancer Center
Houston, Texas 77030

REPORT DATE: August 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

20010504 111

REPORT DOCUMENTATION PAGE

*Form Approved
OMB No. 074-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE August 2000	3. REPORT TYPE AND DATES COVERED Annual (1 Aug 99 - 31 Jul 00)	
4. TITLE AND SUBTITLE Induction of Apoptosis in Human Breast Cancer by Adenoviral-Mediated Gene Transfer of the Transcription Factor E2F-1		5. FUNDING NUMBERS DAMD17-97-1-7162	
6. AUTHOR(S) Kelly Hunt, M.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas M.D. Anderson Cancer Center Houston, Texas 77030		8. PERFORMING ORGANIZATION REPORT NUMBER	
E-MAIL: khunt@mail.mdanderson.org			
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES This report contains colored photos			
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) We have previously shown that the transcription factor E2F-1 results in apoptotic cell death in breast cancer cells when overexpressed in using an adenoviral-mediated system. In order to determine whether the E2F-1 transgene is effective in sensitizing cells to chemotherapy-induced apoptosis, we utilized a recombinant adenovirus in combination with Taxol or doxorubicin and evaluated our results using a two-dimensional isobologram statistical analysis. We observed marked synergistic growth inhibitory effects in breast cancer cell lines treated with a low-dose of adenovirus E2F-1 and low doses of Taxol or doxorubicin. In conclusion, adenovirus-mediated expression of E2F-1 can inhibit breast cancer cell growth synergistically in combination with low-dose Taxol. This combination of gene therapy and chemotherapy may lower the dose of chemotherapeutic agents necessary in the treatment of breast cancer patients and thus may reduce the adverse effects seen with chemotherapy treatments. We plan to explore this further in an animal model using breast cancer xenografts in nude mice.			
14. SUBJECT TERMS Breast Cancer, Apoptosis, E2F-1, Chemotherapy		15. NUMBER OF PAGES 20	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

Table of Contents

Cover.....	
SF 298.....	
Table of Contents.....	1
Introduction.....	2
Body.....	3-7
Key Research Accomplishments.....	8
Reportable Outcomes.....	9-16
Conclusions.....	17
References.....	18
Appendices.....	None

Introduction

Breast cancer continues to be one of the leading causes of death in American women today. Recent changes in the treatment strategies of breast cancer patients have improved the survival rates in American women who develop breast cancer. We continue to see however, over 45,000 deaths each year from metastatic breast cancer. Systemic therapy has been more frequently employed in the treatment of breast cancer patients and recently the overview analysis published by the Early Breast Cancer Trialists Group has shown that systemic chemotherapy can significantly reduce the risk of recurrence and death from breast cancer in both premenopausal and postmenopausal women¹. Current systemic agents utilized are combination regimens with doxorubicin-based chemotherapy and paclitaxel (Taxol) alone or in combination with Adriamycin. These chemotherapeutic agents result in improvements in overall survival and disease-free survival in breast cancer patients, however they are not without significant morbidity. We have previously shown that the E2F-1 transcription factor can result in apoptotic cell death in breast cancer cells *in vitro* when overexpressed using a recombinant adenovirus vector². We have also shown that E2F-1 results in marked apoptotic cell death in breast cancer cells independent of the *p53* status and independent of the *Rb* status. In this report we discuss results obtained in year 3 of this project where we sought to evaluate the results of combination therapies of E2F-1 (AdVE2F) and chemotherapeutic agents on the growth of breast cancer cells *in vitro*. We show here that when E2F-1 is delivered in low-doses it acts synergistically with Taxol and doxorubicin to result in growth inhibition in breast cancer cells.

Body

I. Methods

Three different breast carcinoma cell lines were utilized for the studies described in this report. The three cell lines are MDA-MB-361, MDA-MB-468, and SKBr3. Experiments performed included XTT assay for cell viability, FACS analysis for percent subdiploid cells and analysis of changes in cell cycle profile, and 2-D isobologram analysis to determine the additive or synergistic nature of combination therapies. For the XTT assay cells were added to a 96 well plate at a concentration of 5×10^4 cells/well. After the cells attached to the wells, they were then infected with a recombinant adenovirus expressing the luciferase reporter gene (AdVLuc) or a recombinant adenovirus expressing the E2F-1 gene (AdVE2F) at a multiplicity of infection (MOI) ranging from 10:1 to 100:1. Eight hours following infection with the recombinant adenoviruses, the chemotherapeutic agents were added at different concentrations. Paclitaxel was added at concentrations of 0, 0.1, 0.5, 1, 2.5, 5, 10, and 20 nanomolar. Doxorubicin was utilized at concentrations of 0, 1, 5, 10, 50 and 100 nanomolar. Each of the chemotherapeutics were added to wells with untreated cells (control), wells containing cells with AdVLuc (Luc) and wells containing cells treated with AdVE2F (E2F). XTT assay was performed 24 hours after the addition of the chemotherapeutic agents. Cells were harvested for FACS analysis at 24 hours following treatment with the chemotherapeutics.

II. Results

a. **CELL CYCLE ANALYSIS.** Listed below are the results of cell cycle analysis for each of the three breast carcinoma cell lines after treatment with the recombinant adenoviruses and doxorubicin. Cell cycle changes were most marked in the SKBr3 cell line where we noted a marked increase in the G2/M phase. This was most notable at a doxorubicin concentration of 100 nanomolar. There was a concomitant decrease in the percentage of cells in G1 phase and S phase.

MDA-MB-468	% G1 phase					% G2/M phase					% S phase				
Doxorubicin Concentration	0	1	5	10	50	0	1	5	10	50	0	1	5	10	50
Control	39.1	34.1	22.9	4.9	0.6	22.8	20.6	27.4	33.2	67.7	38.1	45.3	49.7	33.2	31.7
Luc	36.2	37.8	21.9	9.1	1.7	22.6	23.3	35.8	27.4	70.3	41.2	39	42.3	27.4	28
E2F-1(MOI 1:1)	35.8	35.1	25.3	7.3	1.5	23.6	26.6	43.5	55	86	40.6	38.3	43.5	37.7	12.9

MDA-MB-361		% G1 phase						% G2/M phase						% S phase					
Doxorubicin Concentration		0	1	5	10	50	100	0	1	5	10	50	100	0	1	5	10	50	100
Control		48.4	58.7	56.6	56	28.8	23.4	20.2	10.9	11	12.2	28.3	14	31.4	30.3	32.5	31.9	43	62.6
Luc		61.2	58.2	59.4	54.6	31.1	22.3	10.9	10.3	10.3	12	28.8	11.9	27.9	31.5	30.3	33.4	40.1	65.8
E2F-1		62.1	61.7	58.3	57.1	32.8	27.9	10.7	11.3	10.6	13.7	23.8	24.1	27.2	27	31	29.2	43.4	48

SKBr3	G1 phase					% G2/M phase					% S phase				
	0	1	5	10	100	0	1	5	10	100	0	1	5	10	100
Doxorubicin Concentration	55.6	52	49.9	35.3	29.6	13.8	15.8	17.5	37.4	4.6	30.7	32.1	32.6	27.3	65.8
Control	54.9	53.3	48.8	36.8	22.4	12.3	13.4	17.5	34.7	10.2	32.8	33.3	33.7	28.5	67.4
Luc	54.6	56.3	51.5	35	1.8	33.2	33.5	30.7	36.3	68.4	33.2	33.5	30.7	28.7	29.9
E2F-1															

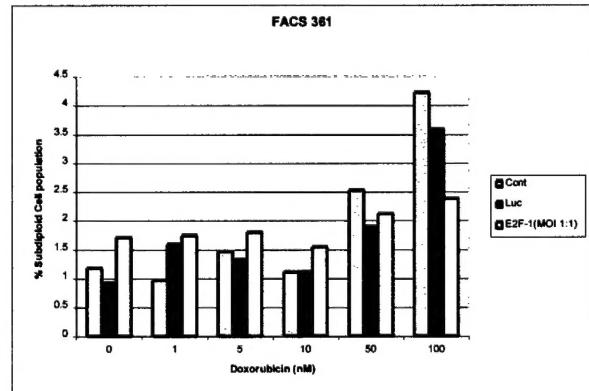
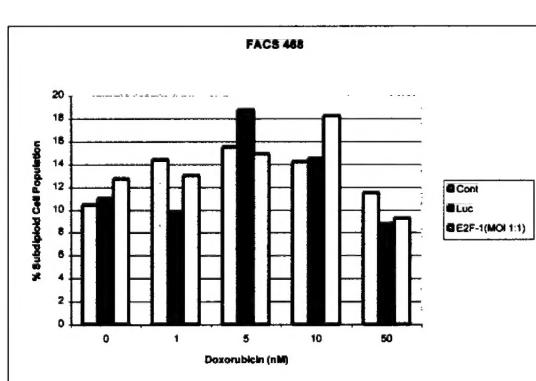
Shown below are the percent changes in cell cycle phases seen in 2 of the breast cancer cell lines following treatment with the recombinant adenoviruses and taxol. Data is shown for the MDA-MB-468 and MDA-MB-361 cell lines. We noted a slight increase in the G2/M phase in the E2F-treated cells.

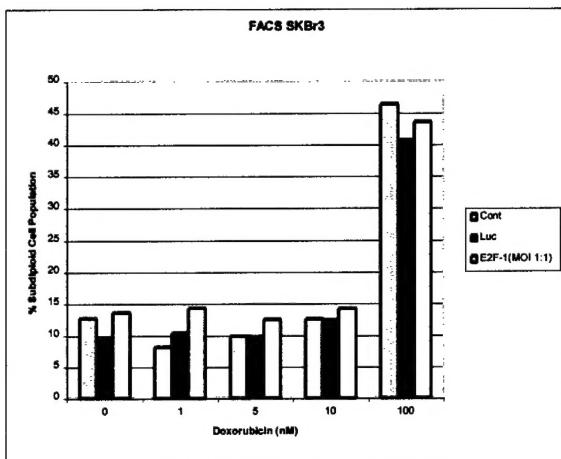
468	% G1 phase				% G2/M phase				% S phase			
	0	0.1	0.5	1	0	0.1	0.5	1	0	0.1	0.5	1
Taxol Concentration	45.3	48	45.3	44.2	19.3	18.1	19.7	18.9	35.4	33.9	35	37
Control	43.7	49.4	47.1	49.9	20.7	18.1	20.1	16	20.7	18.1	20.1	16
Luc	50.5	46.9	52.7	49.7	16.2	17.3	14.4	18	16.2	17.3	14.4	18
E2F-1(MOI 1:1)												

361	% G1 phase						% G2/M phase						% S phase					
	0	0.1	0.5	1	5	10	0	0.1	0.5	1	5	10	0	0.1	0.5	1	5	
Taxol Concentration	49.2	53.5	48.2	50.7	32.1	36.2	18.4	12.2	12.1	12.1	12.2	7.6	32.5	34.3	39.7	37.2	55.7	5
Control	44.7	45.4	41.3	42.2	26	20.9	17.9	16.4	17.7	15	20.3	14.8	37.4	38.2	41	42.8	53.6	6
Luc	53.9	52.4	51	49.7	27.6	20.7	15.7	15.9	14.3	16.5	21.5	23.4	30.4	31.6	34.7	33.8	51	
E2F-1																		

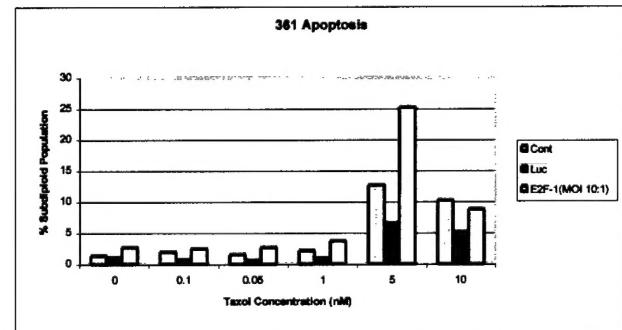
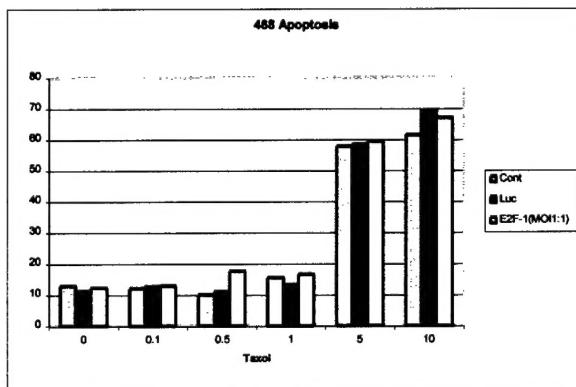
b. ANALYSIS OF SUBDIPLOID CELL POPULATION

Shown below are graphs of the % subdiploid cells following treatment with the recombinant adenoviruses at an MOI of 1:1 followed by treatment with doxorubicin at different concentrations. The cell lines shown are MDA-MB-468, MDA-MB-361, and SKBr3. We did not note a significant increase in subdiploid cells at any of the cell lines except the MDA-MB-468 with doxorubicin concentration at 10 nM. It is possible that we would see an increase in subdiploid cells if we performed the analysis at 48 hours as opposed to 24 hours. We do plan to do these experiments in the near future.





Shown below are graphs of the MDA-MB-468 and MDA-MB-361 cell lines following treatment with the recombinant adenoviruses at an MOI of 1:1 followed by treatment with taxol at varying doses. We did note a significant increase in subdiploid cells in the MDA-MB-361 cell line at a taxol concentration of 5 nM.

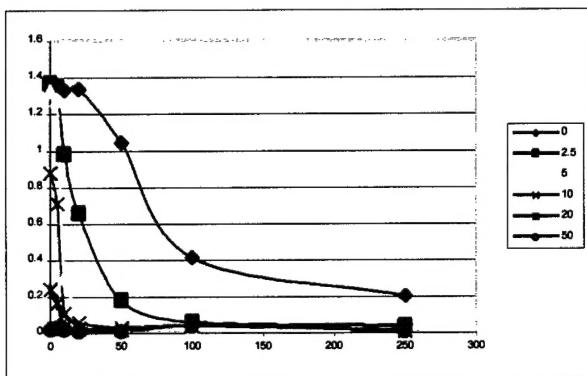


We plan to perform additional assays to evaluate for evidence for apoptotic cell death including TUNEL staining of cytospin preparations and western blot analysis for PARP cleavage. Preliminary cytospin analyses do show an increase in TUNEL positive cells in the breast carcinoma cells pretreated with E2F-1 followed by doxorubicin or taxol.

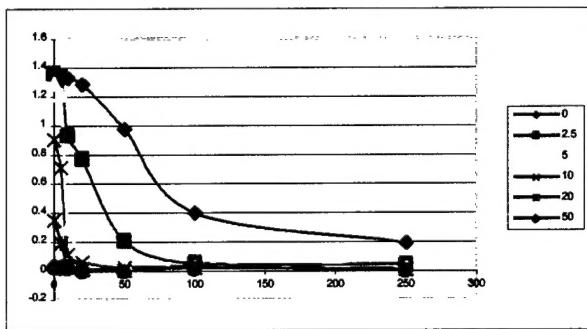
c. Isobolograms

We evaluated all of our combination chemotherapy and recombinant adenoviruses results with 2-D isobolograms and then evaluated for statistical significance using a Wilcoxon Signed-Ranks test. We found that there was synergistic killing when E2F-1 was used in combination with doxorubicin or taxol ($p<0.05$). Shown below are the 2-D isobolograms.

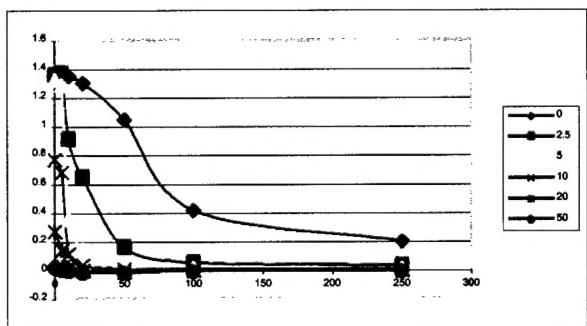
MDA-MB-468 cell line treated with E2F-1 and doxorubicin



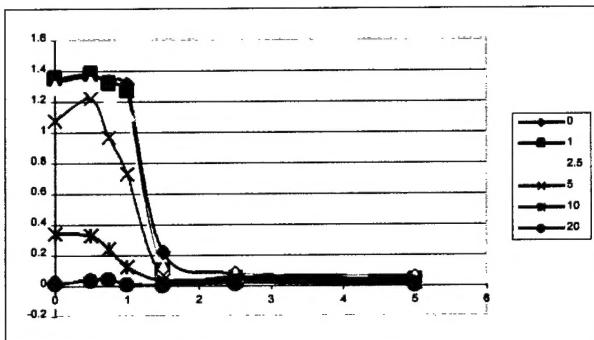
MDA-MB-361 cell line treated with E2F-1 and doxorubicin



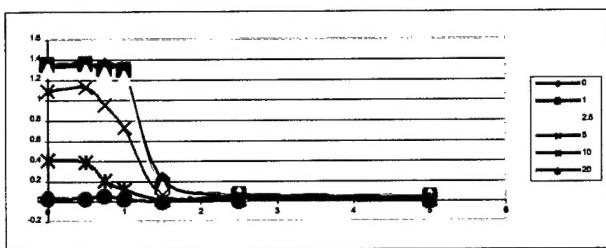
SKBr3 cell line treated with E2F-1 and doxorubicin



MDA-MB-468 cell line treated with E2F-1 and taxol



MDA-MB-361 cell line treated with E2F-1 and taxol



d. Conclusions

Adenovirus mediated overexpression of E2F-1 combined with paclitaxel and doxorubicin results in synergistic cell killing. The synergistic cell killing allows for a decrease in the doses of both the adenovirus E2F-1 and the chemotherapeutic agents. Combining gene therapy strategies with standard chemotherapeutics may improve rates of tumor cell kill and provide alternative treatments for patients who fail standard therapy.

Key Research Accomplishments

- Combination therapy of AdVE2F-1 with taxol and doxorubicin results increased cell death in breast cancer cell in vitro.
- Using a 2-D isobogram method of analysis, we demonstrate here that E2F-1 acts synergistically with taxol and doxorubicin to result in breast cancer cell death.

Reportable Outcomes

1. Hunt KK, Deng J, Liu T-J, Wilson-Heiner M, Swisher SG, Clayman G, Hung M-C. Adenovirus-mediated overexpression of the transcription factor E2F-1 induces apoptosis in human breast and ovarian carcinoma cell lines and does not require p53. *Cancer Research* 1997;57:4722-4726.
2. Bouvet M, Ollila DW, Hunt KK, Babiera GV, Spitz FR, Giuliano AE, Strom AE, Ames FC, Ross MI, Singletary SE. Role of conservation therapy for invasive lobular carcinoma of the breast. *Annals of Surgical Oncology* 1997;4(8):650-654.
3. Ricardo AE, Feig BW, Ellis LM, Hunt KK, Curley SA, MacFadyen BV, Mansfield PF. Gallbladder cancer and trocar site recurrences. *American Journal of Surgery* 1997;174:619-623.
4. Kuerer HM, Newman LA, Buzdar AU, Dhingra K, Hunt KK, Buchholz TA, Binkley SM, Strom EA, Ames FC, Ross MI, Feig BW, McNeese MD, Hortobagyi GN, Singletary SE. Pathologic tumor response in the breast following neoadjuvant chemotherapy predicts axillary lymph node status. *The Cancer Journal from Scientific American* 1998;4(4):230-236.
5. McMasters KM, Giuliano AE, Ross MI, Reintgen DS, Hunt KK, Byrd DR, Klimberg VS, Whitworth PW, Tafra LC, Edwards MJ. Sentinel-lymph-node biopsy for breast cancer - not yet the standard of care. *New England Journal of Medicine* 1998;339(14):990-995.
6. Gershenwald JE, Hunt KK, Kroll S, Ross MI, Baldwin BJ, Feig BW, Ames FC, Schusterman M, Singletary SE. Synchronous elective contralateral mastectomy and immediate bilateral breast reconstruction in women with early-stage breast cancer. *Annals of Surgical Oncology* 1998;5(6):529-538.
7. Hunt KK, Fleming JB, Abramian A, Zhang L, Evans DB, Chiao PJ. Overexpression of the tumor suppressor gene Smad4/DPC4 induces p21^{Waf1} expression and growth inhibition in human carcinoma cells. *Cancer Research* 1998; 58:5656-5661.
8. Newman LA, Kuerer HM, Hunt KK, Kroll SS, Ames FC, Ross MI, Feig BW, Singletary SE. Presentation, treatment, and outcome of local recurrence after skin-sparing mastectomy and immediate breast reconstruction. *Annals of Surgical Oncology* 1998; 5(7):620-626.
9. Kuerer HM, Newman LA, Fornage BD, Dhingra K, Hunt KK, Buzdar AU, Ames FC, Ross MI, Feig BW, Hortobagyi GN, Singletary SE. Role of axillary lymph node dissection after tumor downstaging with induction chemotherapy for locally advanced breast cancer. *Annals of Surgical Oncology* 1998; 5(8):673-680.
10. Kuerer HM, Newman LA, Hunt KK, Dhingra K, Buzdar AU, Binkley SM, Ames FC, Ross MI, Feig BW, Hortobagyi GN, Singletary SE. Residual metastatic axillary lymph nodes following neoadjuvant chemotherapy predict disease-free survival in patients with locally advanced breast cancer. *American Journal of Surgery* 1998; 176:502-509.
11. Heaton KM, Peoples GE, Singletary SE, Feig BW, Ross MI, Ames FC, Buchholz TA, Strom EA, McNeese MD, Hunt KK. Feasibility of breast conservation therapy in metachronous or synchronous bilateral breast cancer. *Annals of Surgical Oncology* 1999;6(1):102-108.

12. Pearlstone DB, Janjan NA, Feig BW, Yasko A, **Hunt KK**, Pollock RE, Lawyer A, Horton J, Pisters PWT. Re-resection with brachytherapy for locally recurrent soft tissue sarcoma arising in a previously radiated field. *The Cancer Journal from Scientific American* 1999;5(1):26-33.
13. Kuerer HM, Newman LA, Smith TL, Ames FC, **Hunt KK**, Dhingra K, Theriault RL, Singh G, Binkley S, Sniege N, Buchholz TA, Ross MI, McNeese MD, Buzdar AU, Hortobagyi GN, Singletary SE. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. *Journal of Clinical Oncology* 1999; 17(2):460-469.
14. Pearlstone DB, Pisters PWT, Bold RJ, Feig BW, **Hunt KK**, Yasko AW, Patel S, Pollack A, Benjamin RS, Pollock RE. Patterns of recurrence in extremity liposarcoma: implications for staging and follow-up. *Cancer* 1999; 85(1):85-92.
15. Newman LA, Kuerer HM, **Hunt KK**, Singh G, Ames FC, Feig BW, Ross MI, Taylor SE, Singletary SE. Local recurrence and survival in black women with early stage breast cancer treated with breast conservation therapy or mastectomy. *Annals of Surgical Oncology* 1999;6(3):241-248.
16. Buchholz TA, **Hunt KK**, Amosson CA, Tucker SL, Strom EA, McNeese MD, Buzdar AU, Singletary SE, Hortobagyi GN. Sequencing of chemotherapy and radiation in lymph node negative breast cancer. *The Cancer Journal from Scientific American* 1999;5(3):159-164.
17. Steck K, **Hunt KK**, Tucker S, Singletary S, El-Naggar AK. Flow cytometric analysis of *KI-67* in invasive ductal carcinoma. Correlation with tumor and patient characteristics. *Oncology Reports* 1999;6:835-838.
18. Kuerer HM, Sahin AA, **Hunt KK**, Newman LA, Breslin TM, Ames FC, Ross MI, Buzdar AU, Hortobagyi GN, Singletary SE. Incidence and impact of documented eradication of breast cancer axillary lymph node metastases prior to surgery in patients treated with neoadjuvant chemotherapy. *Annals of Surgery* 1999;230:72-78.
19. Feig BW, Xiaolin Lu, **Hunt KK**, Shan Q, Yu D, Pollock RE, Chiao P. Inhibition of the transcription factor NF- κ B by adenoviral-mediated expression of I κ B α M results in tumor cell death. *Surgery* 1999;126(2):399-405.
20. Fleming JB, Berman RS, Cheng S-C, Chen NP, **Hunt KK**, Feig BW, Respondek PM, Yasko AW, Pollack A, Patel SR, Burgess MA, Papadopoulos NE, Plager C, Zagars G, Benjamin RS, Pollock RE, Pisters PWT. Long-term outcome of patients with AJCC stage IIB extremity soft tissue sarcomas. *Journal of Clinical Oncology* 1999;17(9):2772-2780.
21. Chiao PJ, **Hunt KK**, Grau AM, Abramian A, Fleming J, Zhang W, Breslin T, Abbruzzese JL, Evans DB. Tumor suppressor gene Smad4/DPC4, its downstream target genes, and regulation of cell cycle. *Annals of the New York Academy of Sciences* 1999;880:31-37.
22. Buzdar AU, Singletary SE, Theriault RL, Booser DJ, Valero V, Ibrahim N, Smith TL, Asmar L, Frye D, Manuel N, Kau S-W, McNeese M, Strom E, **Hunt K**, Ames F, Hortobagyi GN.

Prospective evaluation of paclitaxel versus combination chemotherapy with flourouracil, doxorubicin, cyclophosphamide (FAC) as neoadjuvant therapy in patients with operable breast cancer. *Journal of Clinical Oncology* 1999;17(11):3412-17.

23. Newman LA, Kuerer HM, **Hunt KK**, Ames FC, Ross MI, Theriault R, Fry N, Kroll SS, Robb GL, Singletary SE. Feasibility of immediate breast reconstruction for locally advanced breast cancer. *Annals of Surgical Oncology* 1999;6:671-675.
24. Lenert JT, Vlastos G, Mirza NQ, Winchester DJ, Binkley SM, Ames FC, Ross MI, Feig BW, **Hunt KK**, Strom E, Buzdar AU, Hortobagyi GN, Singletary SE. Primary tumor response to induction chemotherapy as a predictor of histological status of axillary nodes in operable breast cancer patients. *Annals of Surgical Oncology* 1999;6(8):762-767.
25. Newman LA, Kuerer HM, **Hunt KK**, Laronga CL, Breslin T, Singletary SE. Hormonal and nutritional considerations in breast cancer risk and survival. *Journal of Surgical Oncology* 1999;71:250-260.
26. Pataer A, Fang B, Yu R, Kagawa S, **Hunt KK**, McDonnell TJ, Roth JA, Swisher SG. Adenoviral Bak overexpression mediates caspase-dependent tumor killing. *Cancer Research* 2000; 60(4):788-92.
27. Vlastos G, Mirza NQ, Lenert JT, **Hunt KK**, Ames FC, Feig BW, Ross MI, Buzdar AU, Singletary SE. The feasibility of minimally invasive surgery in stage IIA, IIB, and IIIA breast carcinoma patients after tumor downstaging with induction chemotherapy. *Cancer* 2000;88(6):1417-1424.
28. Kuerer HM, **Hunt KK**, Newman LA, Ross MI, Ames FC, Singletary SE. Neoadjuvant chemotherapy in women with invasive breast carcinoma: conceptual basis and fundamental surgical issues. *Journal of the American College of Surgeons* 2000;190(3):350-363.
29. Peng H, Xu F, Pershad R, **Hunt KK**, Frazier ML, Berchuck A, Gray JW, Hogg D, Bast RC Jr, Yu Y. ARHI is the center of allelic deletion on chromosome 1p31 in ovarian and breast cancers. *International Journal of Cancer* 2000;86:690-694.
30. Meric F, Mirza NQ, Buzdar AU, **Hunt KK**, Ames FC, Ross MI, Pollock RE, Newman LA, Feig BW, Strom EA, Buchholz TA, McNeese MD, Hortobagyi GN, Singletary SE. Prognostic implications of pathological lymph node status after preoperative chemotherapy for operable T3N0M0 breast cancer. *Annals of Surgical Oncology* 2000;7(6):435-440.
31. Meric F, Milas M, **Hunt K**, Hildebrandt G, Hess K, Pisters PWT, Papadopoulos N, Plager C, Burgess M, Patel P, Benjamin RS, Pollock RE, Feig BW. Impact of neoadjuvant chemotherapy on postoperative morbidity in soft-tissue sarcomas. *Journal of Clinical Oncology* (In press).
32. Cohen L, Breslin T, Kuerer H, Ross M, **Hunt K**, Sahin A. Identification and evaluation of axillary sentinel lymph nodes in patients with breast carcinoma treated with neoadjuvant chemotherapy. *American Journal of Surgical Pathology* (In press).
33. Freedman LM, Buchholz TA, Thames HD, Strom EA, McNeese MD, Hortobagyi GN, Singletary SE, Heaton KM, **Hunt KK**. Local-regional control in breast cancer patients with a presumed

genetic predisposition. *International Journal of Radiation Oncology, Biology and Physics* 2000 (In press).

34. Newman LA, Kuerer HM, Hunt KK, Vlastos G, Ames FC, Ross MI, Singletary SE. Prophylactic mastectomy. *Journal of the American College of Surgeons* (In press).
35. Yoshida K, Feig BW, Lu XL, Chiao PJ, Hunt KK. Adenovirus-mediated overexpression of IkBaM induces apoptosis in breast cancer cell lines [Abstract]. Proceedings of the American Association for Cancer Research, March 1999;40:487(#3216).
36. Lenert J, Buzdar A, Winchester E, Strom F, Ames F, Hunt K, Feig B, Singletary E. Substitution of axillary irradiation (AXRT) for axillary lymph node dissection (ALND) based on primary tumor's response to induction chemotherapy (ICT) [Abstract]. Proceedings of the Society of Surgical Oncology 52nd Annual Cancer Symposium, March 1999; pp. 10 (Abstract #8).
37. Yoshida K, Liu T-J, Xia W, Goodrich D, Wilson-Heiner M, Abramian A, Feig B, Hunt K. Truncated mutant retinoblastoma gene induces apoptosis in breast cancer cells *in vitro* and *in vivo*. Proceedings of the 22nd Annual San Antonio Breast Cancer Symposium. *Breast Cancer Research and Treatment* 57(1):94 (Abstract #369), 1999.
38. Newman L, Kuerer H, Hunt K, Ames F, Ross M, Theriault R, Fry N, Kroll S, Robb G, Singletary E. Feasibility of immediate breast reconstruction (IBR) for locally advanced breast cancer (LABC) [Abstract]. Proceedings of the Society of Surgical Oncology 52nd Annual Cancer Symposium, March 1999;Plenary Session, (Abstract #30).
39. Mirza N, Singletary E, Kemp B, Buchholz T, Ames F, Ross M, Feig B, Hunt K. Mucinous carcinoma of the breast: Is lumpectomy alone adequate therapy? [Abstract]. Proceedings of the Society of Surgical Oncology 52nd Annual Cancer Symposium, March 1999, pp.41(#P28).
40. Breslin T, Cohen L, Fleming J, Kuerer H, Delpassand E, Colchin R, Feig B, Ames F, Sahin A, Ross M, Hunt K. Lymphatic mapping and sentinel node biopsy is accurate following preoperative chemotherapy for breast cancer. Proceedings of the Society of Surgical Oncology 52nd Annual Cancer Symposium, March 1999: Poster Presentation, (#P29).
41. Portera C Jr, Ho V, Respondek P, Hunt K, Feig B, Patel S, Pollack A, Yasko A, Pollock R, Pisters PWT. Alveolar soft part sarcoma (ASPS): Long-term results from 70 consecutive patients treated at a single institution. Proceedings of the Society of Surgical Oncology 52nd Annual Cancer Symposium, March 1999; Poster Presentation, pp.61(#P107).
42. Newman LA, Kuerer HM, Hunt KK, Ross MI, Ames FC, Feig BW, Buzdar AU, Fornage B, Singletary SE. Adverse prognostic impact of infraclavicular lymph nodes detected by ultrasound in patients with locally advanced breast cancer (LABC). Proceedings of the American Society of Clinical Oncology, page 71a (Abstract #267), 1999.
43. Buzdar AU, Hortobagyi GN, Theriault RL, Booser DJ, Valero VV, Ibrahim NI, Asmar L, Frye D, Manuel N, Kau S, McNeese M, Strom E, Hunt K, Singletary E, Ames F. Prospective randomized trial of taxol (Tax) alone versus flourouracil, doxorubicin, cyclophosphamide (FAC) as an

induction therapy in patients with operable breast cancer (Br Ca). Proceedings of the American Society of Clinical Oncology, page 73a (Abstract #273), 1999.

44. Dhingra K, Esparza-Guerra L, Valero V, Booser D, Theriault R, Singletary E, Strom E, McNeese M, Buzdar A, Sahin A, **Hunt K**, Martinez N, Hortobagyi G. A Phase III randomized trial of dose-intensive, neoadjuvant 5FU, doxorubicin, cyclophosphamide (FAC) with G-CSF (filgrastim) in locally advanced breast cancer (LABC)-efficacy and safety data. Proceedings of the American Society of Clinical Oncology, page 74a (Abstract #278), 1999.
45. Fleming J, Breslin T, Delpassand A, Whitman G, Fornage B, Sahin A, Ames F, Feig B, Ross M, **Hunt K**. The utility of preoperative lymphoscintigraphy in the management of early stage breast cancer patients receiving sentinel node biopsy. Proceedings of the American Society of Clinical Oncology, page 76a (Abstract #286), 1999.
46. Patel SR, Jenkins J, Papadopoulos NE, Burgess MA, Plager C, Pisters PWT, Feig BW, **Hunt K**, Pollack A, Benjamin RS. Preliminary results of a two-arm phase 2 trial of gemcitabine (Gem) in patients (Pts) with gastrointestinal leiomyosarcomas (leios) and other soft tissue sarcomas (STS). Proceedings of the American Society of Clinical Oncology, page 541a (Abstract #2091), 1999.
47. Meric F, Milas M, **Hunt K**, Hildebrandt G, Hess K, Pisters PWT, Patel S, Benjamin RS, Pollock RE, Feig BW. Impact of neoadjuvant chemotherapy on postoperative morbidity in sarcomas. Proceedings of the American Society of Clinical Oncology, page 546a (Abstract #2109), 1999.
48. Salem N, **Hunt KK**, Meistrich ML, Meyn R, Pollack A. Adenoviral-mediated E2F-1 expression sensitizes prostate cancer cells in vitro to ionizing radiation. [Abstract]. (Accepted for oral presentation at the 1999 Annual Meeting of The American Society For Therapeutic Radiology and Oncology, November 1999). In Press, Cancer Journal of Scientific American.
49. Freedman LM, Thames HD, **Hunt KK**, Strom EA, McNeese MD, Heaton KM, Buchholz TA. Local-regional control in breast cancer patients with a presumed genetic predisposition. [Abstract]. Proceedings of the Annual Meeting of The American Society For Therapeutic Radiology and Oncology, November 1999.
50. Holtz D, **Hunt K**, Bilimoria M, Mirza N, Feig B, Pisters P, Benjamin R, Patel S, Papadopoulos N, Plegar C, Burgess M, Pollock R, Plager C. Recent experience with sarcomatosis: Low volume disease predicts for improved survival. Proceedings of the 5th Annual Scientific Meeting of the Connective Tissue Oncology Society, Washington, D.C., October, 1999.
51. Meric F, Milas M, **Hunt K**, Hildebrandt G, Hess K, Pisters PWT, Papadopoulos N, Plager C, Burgess M, Patel P, Benjamin RS, Pollock RE, Feig BW. Impact of neoadjuvant chemotherapy on postoperative morbidity in soft-tissue sarcomas. Proceedings of the 5th Annual Scientific Meeting of the Connective Tissue Oncology Society, Washington, D.C., October, 1999.
52. Abramian AJ, Yoshida K, Mirza NQ, Liu T-J, **Hunt KK**. Increased rates of cell death by combining adenoviral-mediated expression of E2F-1 with paclitaxel in human breast cancer cell lines. Proceedings of the 22nd Annual San Antonio Breast Cancer Symposium. Breast Cancer Research and Treatment 57(1):54 (Abstract #179), 1999.

53. Vlastos G, Mirza NQ, Lenert JT, Hunt KK, Ames FC, Feig BW, Ross MI, Buzdar AU, Singletary SE. Feasibility of minimally invasive surgery in stage IIA, IIB, and IIIA breast cancer patients after tumor downstaging with induction chemotherapy. Proceedings of the 22nd Annual San Antonio Breast Cancer Symposium. *Breast Cancer Research and Treatment* 57(1):110 (Abstract #451), 1999.
54. Meric F, Mirza NQ, Vlastos G, Ames FC, Feig BW, Ross MI, Pollock RE, Buchholz TA, Strom EA, McNeese MD, Hortobagyi GN, Singletary SE, Hunt KK. Breast conservation surgery: Long-term results from a single institution. Proceedings of the 22nd Annual San Antonio Breast Cancer Symposium. *Breast Cancer Research and Treatment* 57(1):51 (Abstract #165), 1999.
55. Mirza NQ, Vlastos G, Meric F, Singletary E, Reed L, Ames FC, Feig BW, Ross MI, Pollock RE, Buchholz TA, Strom EA, McNeese MD, Theriault R, Buzdar AU, Hortobagyi GN, Hunt KK. Time to local-regional recurrence (LRR) following breast conserving therapy (BCT) predicts survival. Proceedings of the 22nd Annual San Antonio Breast Cancer Symposium. *Breast Cancer Research and Treatment* 57(1):49 (Abstract #160), 1999.
56. Newman LA, Ferry P, Hunt KK, Robinson A, LaRonga C, Singletary SE. Efficacy of mammographic screening in a predominantly young minority-ethnicity population: Results of a community-based cancer care initiative. Proceedings of the 22nd Annual San Antonio Breast Cancer Symposium. *Breast Cancer Research and Treatment* 57(1):47 (Abstract #149), 1999.
57. Yoshida K, Liu T-J, Abramian A, Wilson-Heiner M, Goodrich D, Hunt K. Adenovirus-mediated overexpression of a truncated retinoblastoma gene product induces apoptosis via enhanced E2F-1 expression [Abstract]. Proceedings of the Society of Surgical Oncology 52nd Annual Cancer Symposium, March 1999; pp.15 (Abstract #28).
58. Janjan N, Crane CH, Ajani J, Goswitz M, Hunt K, Feig B, Chapman J, Rich TA, Skibber J, Dong L. 30 Gy is a sufficient dose to control micrometastases from anal cancer: Dosimetric evaluation of N0 patients undergoing chemoradiation [Abstract]. Proceedings of the American Radium Society 81st Annual Meeting, April 1999; pp. 117 (Abstract #11).
59. Meric F, Mirza NQ, Buzdar AU, Hunt KK, Ames FC, Ross MI, Strom EA, Pollock RE, Newman LA, Feig BW, Fryer D, Buchholz TA, McNeese MD, Hortobagyi GN, Singletary SE. Prognostic implications of lymph node status after neoadjuvant chemotherapy (NCT) for operable T3N0 breast cancer. [Abstract] Proceedings of the 53rd Annual Cancer Symposium of the Society of Surgical Oncology, March 2000, pp. 12 (Abstract #16).
60. Yoshida K, Liu T-J, Goodrich B, Feig B, Hunt K. Adenovirus mediated overexpression of truncated Rb gene enhances the effect of chemotherapeutic agents on breast carcinoma cell lines. [Abstract] Proceedings of the 53rd Annual Cancer Symposium of the Society of Surgical Oncology, March 2000, pp. 20 (Abstract #47).
61. Bilimoria MM, Evans DB, Lee JE, Berman RS, Feig BW, Hunt KK, Mansfield PF, Pollock RE, Pisters PWT. Pancreatic closure after distal pancreatectomy: Relationship to postoperative pancreatic fistula. [Abstract] Proceedings of the 53rd Annual Cancer Symposium of the Society of Surgical Oncology, March 2000, pp. 24 (Abstract #63).

62. Mirza N, Vlastos G, Singletary E, Ames F, Ross M, Feig B, Pollock R, Buchholz T, **Hunt KK**. Ductal carcinoma *in situ* (DCIS): Long-term results of breast conserving therapy (BCT). [Abstract] Proceedings of the 53rd Annual Cancer Symposium of the Society of Surgical Oncology, March 2000, pp. 27 (Abstract #74).

63. Dackiw APB, Meric F, Chang DW, Colchin M, Porter GA, Ross MI, **Hunt KK**, Pollock RE, Ames FC, Feig BW, Newman LA, Robb GL, Singletary SE. Incidence and outcome of contralateral breast cancer (CBC) in patients status post unilateral mastectomy (MRM) with unilateral breast reconstruction (UBR). Proceedings of the 53rd Annual Cancer Symposium of the Society of Surgical Oncology, March 2000. Poster Presentation, pp.42 (#P29).

64. Yoshida K, Feig, B, Chiao PJ, Lu XL, **Hunt KK**. Adenovirus mediated overexpression of mutant I κ B α M gene enhances the effect of chemotherapeutic agents on the MCF-7 breast carcinoma cell line. [Abstract] Proceedings of the 91st Annual Meeting of the American Association for Cancer Research, April 2000, pp. 350 (Abstract #2222).

65. Abujaiang P, Fang B, Yu R, Kagawa S, **Hunt KK**, McDonnell TJ, Roth JA, Swisher SG. Adenoviral bak overexpression mediates caspase dependent tumor killing. [Abstract] Proceedings of the 91st Annual Meeting of the American Association for Cancer Research, April 2000, pp. 731 (Abstract #4644).

66. Buchholz TA, Tucker SL, Mathur D, Strom EA, McNeese MD, Hortobagyi GN, Cristoforilli M, Esteva F, Newman L, Singletary SE, **Hunt KK**. Impact of systemic treatment on local control for lymph-node negative breast cancer patients treated with breast conservation therapy. [Abstract] Accepted for oral presentation at the Annual Meeting of The American Radium Society, London, England, April 2000.

67. Swisher SG, Pataer A, Fang B, Yu R, Nishizaki M, Fang B, **Hunt KK**, Roth JA. Enhancement of adenoviral mediated *p53* (*Adp53*) tumor killing with amifostine induced CDC2 kinase activation. [Abstract] Surgical Forum April 2000 (In press).

68. Pisters P, Patel S, Crane C, Feig B, **Hunt K**, Burgess M, Papadopoulos N, Plager C, Benjamin R, Pollock R, Janjan N. Phase I trial of preoperative doxorubicin-based concurrent chemoradiation and electron-beam intraoperative radiation therapy (IORT) for resectable retroperitoneal sarcomas. [Abstract] Proceedings of the American Society of Clinical Oncology (Abstract #2199) 2000.

69. Porter G, Ahmad S, Cantor S, Lenert J, **Hunt K**, Feig B, Patel S, Benjamin R, Pollock R, Pisters P. Cost-effectiveness of routine chest computed tomography in patients with T2 soft-tissue sarcoma. [Abstract accepted] American Society of Clinical Oncology 2000.

70. Meric F, Hess KR, Varma DGK, **Hunt KK**, Pisters PWT, Milas M, Patel SR, Benjamin, Plager C, Papadopoulos NEJ, Burgess MA, Pollock RE, Feig BW. Impact of neoadjuvant therapy on local control of soft tissue sarcomas. [Abstract accepted] American Society of Clinical Oncology.

71. Ahmad S, Patel S, Baker T, Feig B, **Hunt K**, Yasko A, Burgess M, Papadopoulos N, Plager C, Benjamin R, Pollock R, Pisters P. Extraosseous osteosarcoma: Long-term results from a single institution experience. [Abstract accepted] American Society of Clinical Oncology 2000.

72. Wayne JD, Langstein HN, Pollack A, Hunt KK, Feig BW, Yasko AW, Smith LG, Zagars GK, Pollock RE, O'Sullivan B, Pisters PW. Preoperative radiotherapy for extremity soft tissue sarcoma (STS): Site-specific wound complication rates and the impact of reconstructive surgery. [Abstract accepted] American Society of Clinical Oncology 2000.

Conclusions

Adenovirus mediated overexpression of E2F-1 combined with paclitaxel and doxorubicin results in synergistic cell killing. The synergistic cell killing allows for a decrease in the doses of both the adenovirus E2F-1 and the chemotherapeutic agents. Combining gene therapy strategies with standard chemotherapeutics may improve rates of tumor cell kill and provide alternative treatments for patients who fail standard therapy.

References

1. Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. *Lancet* 1998;352:930-942.
2. Hunt KK, Deng J, Liu T-J, Wilson-Heiner M, Swisher SG, Clayman G, Hung M-C. Adenovirus-mediated overexpression of the transcription factor E2F-1 induces apoptosis in human breast and ovarian carcinoma cell lines and does not require p53. *Cancer Research* 1997;57:4722-4726.